The opinion in support of the decision being entered today was *not* written for publication and is *not* binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte PAUL K. WOLBER, ROBERT H. KINCAID
DOUGLAS A. AMORESE, DIANE D. ILSLEY and
ANDREW S. ATWELL

Appeal 2006-1553
Application 09/628,472
Technology Center 1600

Decided: May 21, 2007

Before TONI R. SCHEINER, DONALD E. ADAMS, and LORA M. GREEN, *Administrative Patent Judges*.

GREEN, *Administrative Patent Judge*.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the Examiner's final rejection of claims 1-15 and 21-23. We have jurisdiction under 35 U.S.C. § 6(b). Claims 1, 10, 13, and 23 are representative of the claims on appeal, and read as follows:

1. A method for producing a mixture of nucleic acids, said method comprising:

(a) providing an array of distinct single-stranded probe nucleic acids of differing sequence immobilized on a substrate where each distinct probe present on said array comprises a constant domain and a complement variable domain; wherein said complement variable domain is at the 5' end of each distinct probe;

(b) hybridizing nucleic acids complementary to said constant domain with said array of single-stranded probe nucleic acids to produce a template array of overhang comprising duplex nucleic acids, wherein each overhang comprising duplex nucleic acid of said array comprises a double-stranded constant region and a single-stranded variable region overhang;

(c) subjecting said template array of overhang comprising duplex nucleic acids to a cyclic reaction that produces a mixture of linearly amplified amounts of single stranded nucleic acids of differing sequence; and

(d) separating said mixture of nucleic acids from said template array.

10. A method of making a population of target nucleic acids from an initial mRNA sample, said method comprising:

(a) generating a mixture of nucleic acids according to the method of Claim 1; and

(b) employing said mixture of nucleic acids as primers in a target generation step in which target nucleic acids are produced from said mRNA sample;

whereby said population of target nucleic acids is produced.

13. A hybridization assay comprising the steps of:

(a) generating a set of target nucleic acids according to the method of Claim 10;

(b) contacting said set of target nucleic acids with an array of probe nucleic acids under hybridization conditions; and

(c) detecting the presence of target nucleic acids hybridized to probe nucleic acids of said array.

23. A method for producing a mixture of nucleic acids, said method comprising:

(a) providing an array of distinct single-stranded probe nucleic acids of differing sequence where each distinct probe present on said array comprises a constant domain and a complement variable domain; wherein said complement variable domain is at the 5' end of said each distinct probe;

(b) hybridizing nucleic acids complementary to said constant domain with said array of single-stranded probe nucleic acids to produce a template array of overhang comprising duplex nucleic acids, wherein each overhang comprising duplex nucleic acid of said array comprises a double-stranded constant region and a single-stranded variable region overhang;

(c) subjecting said template array of overhang comprising duplex nucleic acids to a strand displacement amplification protocol to produce a mixture of single stranded nucleic acids of differing sequence; and

(d) separating said mixture of nucleic acids from said template array.

Claims 1-9, 21, and 22 stand rejected under 35 U.S.C. § 103(a) as being obvious over the combination of Dattagupta '363[1] and Conrad.[2] Claims 10-15 stand rejected under 35 U.S.C. § 103(a) as being obvious over the previous combination as further combined with Dattagupta '899.[3] Finally, claim 23 stands rejected under 35 U.S.C. § 103(a) as being obvious over the combination of Dattagupta '363, Conrad, and Dattagupta '899. We affirm-in-part.

BACKGROUND

The Specification states in the section entitled "Background of the Invention," that:

> The characterization of cellular gene expression (i.e. gene expression analysis) finds application in a variety of disciplines,

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[1] Dattagupta, U.S. patent No. 4,734,363, issued March 29, 1988.
[2] Conrad, U.S. Patent No. 5,652,099, issued July 29, 1997.
[3] Dattagupta, U.S. Patent No. 5,215,899, issued June 1, 1993.

> such as in the analysis of differential expression between different tissue types, different stages of cellular growth or between normal and diseased states.
>
> * * *
>
> A fundamental step in gene expression analysis assays is . . . the step of labeled target generation. Target generation protocols typically include a primer extension reaction, in which a primer is contacted with an initial mRNA sample to produce a labeled target population . . . . In . . . [some] protocols, custom primer mixes are employed in target generation. While such protocols overcome . . . disadvantages with poly A and random primer based protocols, custom primer mix or gene specific primer based protocols can be prohibitively expensive, particularly in array-based hybridization protocols in which custom arrays are employed.

(Specification, pages 1-2.)

The Specification then states that "there is continued interest in the development of new primer generation protocols," and that the invention is drawn to "[m]ethods for generating mixtures of nucleic acids." (*Id.* at 2.)

DISCUSSION

Claims 1-9, 21, and 22 stand rejected under 35 U.S.C. § 103(a) as being obvious over the combination of Dattagupta '363 and Conrad. As Appellants do not argue the claims separately, we will focus our analysis on independent claim 1. 37 C.F.R. § 41.37(c)(1)(vii) (2006).

Dattagupta '363 is relied upon for teaching "a method for producing a mixture of nucleic acid comprising providing distinct single-stranded nucleic acids immobilized on a substrate wherein the nucleic acid comprises a constant domain at the 3' end (complement to #16, Fig. 1) and a variable region at the 5' end (complement to #20, Fig. 1), hybridizing a nucleic acid (#16) to the constant domain to produce a duplex having a double-stranded

region and a single-stranded variable-region overhang, subjecting the duplex to cyclic reactions to produce a linearly amplified single-stranded nucleic acids and separating the single-stranded nucleic acids (column 1, line 65-Column 2, line 58 and Examples 1, 5 and 6)." (Answer 3-4.)

Conrad is relied upon for teaching "a method for producing a mixture of nucleic acids the method comprising providing a plurality of different sequence single-stranded nucleic acids, each comprising a constant domain at the 3' end (e.g. M13 primer site (fig. 14) and/or T7/SP6 promoter sites (fig. 23)) and a variable domain at the 5' end (i.e. vector insert and/or gene template) hybridizing nucleic acids to the constant domain to produce a duplex having a double-stranded region and a single-stranded variable-region overhang and subjecting the duplex to cyclic reactions to produce a mixture of linearly-amplified single stranded nucleic acids of differing sequences (i.e. probe cocktail) (Example 2, Column 24, line 53-Column 26, line 3)." (*Id.* at 4.)

The Examiner concludes:

> Dattagupta ['363] does not teach the immobilized nucleic acids have differing sequences. However, as detailed above, Conrad teaches the similar method using nucleic acids of differing sequences whereby a "cocktail of probes" is produced for detection of differing length, different number and/or different location of targets. Conrad further teaches these three different types diagnostic targets illustrate the broad importance of the probe cocktail producing [sic] (Column 28, lines 50-58). It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the probe synthesis of Dattagupta ['363] by immobilizing additional nucleic acids of different sequences for the expected benefit of proving various types of diagnostics based and further based on the broad importance of probe cocktails as taught by Conrad (Column 28, lines 50-58).

> Conrad does not teach immobilized duplexes. However, as detailed above, Dattagupta ['363] teach[es] the similar method wherein the duplexes are covalently immobilized (Fig. 1). Dattagupta ['363] teaches their immobilized duplexes provide large-scale production of sequence specific probes while eliminating the need for plasmids, cloning and restriction (Column 1, lines 31-35). It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the probe synthesis of Conrad by immobilizing their plurality of duplexes for the expected benefit of providing large scale production of sequence-specific probes as taught by Dattagupta ['363] (Column 1, lines 31-35).

(*Id.* at 4-5.)

"In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a prima facie case of obviousness. Only if that burden is met, does the burden of coming forward with evidence or argument shift to the applicant." *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993) (citations omitted). In order to determine whether a prima facie case of obviousness has been established, we considered the factors set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1996); (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the relevant art; and (4) objective evidence of nonobviousness, if present.

Claim 1 is drawn to a method for producing a mixture of nucleic acids, comprising the steps of:

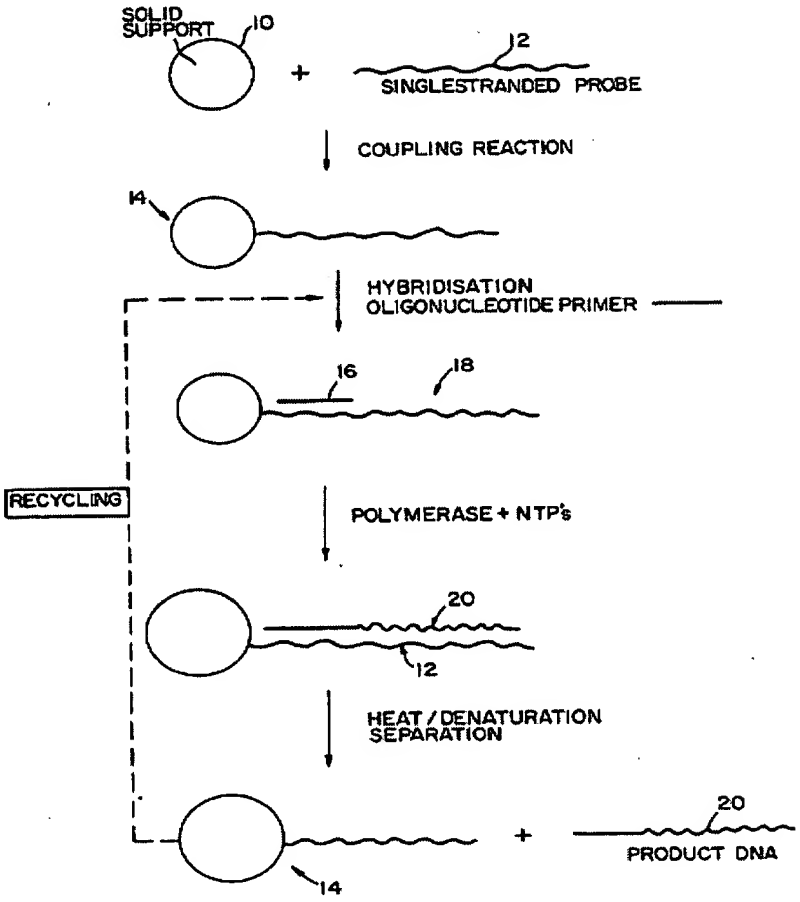
1) providing an array of distinct single-stranded probe nucleic acids of differing sequence immobilized on a substrate where each distinct probe present on said array comprises a constant domain and a complement variable domain; wherein said complement variable domain is at the 5' end of each distinct probe;

2) hybridizing nucleic acids complementary to said constant domain with said array of single-stranded probe nucleic acids to produce a template array of overhang comprising duplex nucleic acids, wherein each overhang comprising duplex amino acid of said array comprises a double-stranded constant region and a single-stranded variable region overhang;

3) subjecting said template array of overhang comprising duplex nucleic acids to a cyclic reaction that produces a mixture of linearly amplified amounts of single stranded nucleic acids of differing sequences; and

4)separating said mixture of nucleic acids from said template array.

Dattagupta '363, as noted by the Examiner, teaches a method of production of a single stranded nucleic acid probe (Abstract). Figure 1 of Dattagupta '363, relied upon by the Examiner in the rejection, shows a method of synthesis of single stranded nucleic acids, and is set forth below:



In the method, a single-stranded DNA complementary to the strand to be synthesized (**12**) is coupled by its 3' to a solid support (**10**) (Dattagupta '363, col. 1, ll. 65-68). An oligonucleotide corresponding to the 5' end of the desired strand (**16**) is then hybridized to strand (**12**) (the portion of (**12**) to which (**16**) hybridizes to reads on the constant domain, leaving a variable region overhang), which is then contacted with a solution containing DNA polymerase and NTPs, elongating (**16**) at its 3' end using (**12**) as a template, producing the desired single stranded DNA product (**20**) (*id.* at col. 2, ll. 1-9). After being subjected to denaturing conditions, the product (**20**) is

released into solution, and can be separated from the solid support and recovered from solution in any known manner (*id.* at ll. 10-18). In addition, although Figure 1 illustrates the use of a microbead, Dattagupta '363 teaches that "the solid support can be cellulose, Sephadex, or Sepharose, a piece of paper, nylon or anything which can be used to react with an amine or aldehyde or similar residue." (*Id.* at ll. 19-22).

Thus, Dattagupta '363 teaches the method of synthesis, but does not teach the use of the method to synthesize a mixture of single stranded nucleic acids.

Conrad teaches solution phase synthesis of single stranded nucleic acid probes, in which a constant primer is used (reads on the constant region), in which either only one template strand or both template strands (reads on the variable region) may be transcribed. (Conrad, Example 2, col. 24, l. 53-col. l. 3). Conrad specifically teaches that "[a] mixture of plasmids containing several different plasmids can be used to create a 'cocktail' of linearized templates from which the corresponding "cocktail" of probes . . . which can bind to multiple sites on a genomic sequence, can be concurrently transcribed." (*Id.* at col. 25, l. 65-col. 26, l. 3.)

Thus, we agree with the conclusion of the Examiner that it would have been obvious to the ordinary artisan to use the method of Dattagupta '363 to synthesize a cocktail of probes as taught by Conrad to obtain the advantages of solid phase synthesis methods as taught by Dattagupta '363, that is, easy separation of the synthesized probe from the immobilized template, as well being able to perform large scale production. It would have also been obvious to use a constant primer because Conrad teaches the concept of a primer that can bind to two template strands, and one could

prime all of the templates using a single primer. Finally, immobilizing an array of nucleic acids on a single substrate is well known to the ordinary artisan, and Dattagupta teaches that other substrates such as paper or anything compatible with the chemistry may be used. Thus, the rejection of the Examiner is affirmed.

Appellants argue that the Examiner has used impermissible hindsight in combining the references (Br. 9). Dattagupta '363, Appellants assert, "only describes a single type of template immobilized on a given solid support . . .[which] is an important feature of the asserted utility of the '363 patent in that the structure is a sensitive probe for a single nucleic acid analyte." (Br. 9-10.) Thus, Appellants aver, one would not have been motivated to modify the bead support of Dattagupta '363 to carry more than one template because then one could not use the synthesized primer "to detect a single analyte, but just the presence of at least one of two or more different analytes," and "a proposed modification of a prior art invention 'cannot render the prior art invention being modified unsatisfactory for its intended purpose.'" (Br. 10, citing MPEP § 2143.01.)

Appellants argue further that "one would not be motivated to modify the method disclosed in [Conrad] such that multiple plasmids are replaced with bead bound nucleic acids. One of skill in the art would not be so motivated because access to the polymerase enzymes to the template nucleic acid could potentially be hindered if the template DNA were immobilized on a solid support surface." (Br. 10). Thus, Appellants conclude, "the only motivation that is present to combine the teaching of [Dattagupta '363] and [Conrad] is the present application." (*Id.*)

Appellants' arguments are not convincing. Dattagupta '363 is drawn to a method of synthesizing nucleic acid probes, in which "[o]ne such use if [sic] for making probes for diagnostic tests." [Dattagupta '363, col. 2, ll. 63-64.] Thus, Dattagupta '363 is not drawn to any single use, just a suggested use. In addition, Conrad teaches synthesis of a cocktail of probes and their uses, thus it would have been obvious to the ordinary artisan to use the synthetic method of Dattagupta '363 to synthesize the cocktail of probes as taught by Conrad to obtain the advantages of Dattagupta '363, that is synthesis of large amounts of probe and the well known advantages of solid phase synthetic methods, such as ease of separation of the synthesized probe from the immobilized template. In addition, Dattagupta '363 demonstrates that it is known to synthesize nucleic acids on a solid support without hindering the polymerase enzyme As set forth by the Supreme Court in *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 82 USPQ2d 1385, 1397 (2007),

> In determining whether the subject matter of a patent claim is obvious, neither the particular motivation nor the avowed purpose of the patentee controls. What matters is the objective reach of the claim. If the claim extends to what is obvious, it is invalid under § 103. One of the ways in which a patent's subject mater can be proved obvious is by noting that there existed at the time of invention a known problem for which there was an obvious solution encompassed by the patent's claims.

In this case, the known problem is the generation of a cocktail or mixture of probes, as taught by Conrad, for which there was an obvious solution, the use of solid phase synthetic methods as taught by Dattagupta '363.

Moreover, as to hindsight, the *KSR* Court noted it was aware of the "distortion caused by hindsight bias," but stated that "[r]igid preventative rules that deny fact finders recourse to common sense, however, are neither necessary under our case law nor consistent with it." *Id.* at 1397. Thus, in the case before us, both Dattagupta '363 and Conrad are drawn to the synthesis of single stranded nucleic acid probes, and it would have been obvious to the ordinary artisan to synthesize the cocktail (i.e. mixture) of probes of Conrad using the method of Dattagupta '363 to obtain the advantages of solid phase synthesis taught by Dattagupta '363.

Appellants also argue that Dattgupta '363 does not teach producing a mixture of nucleic acids, and there is no teaching or suggestion in that reference to make a mixture of nucleic acids (Br. 12). Moreover, Appellants assert, claim 1 requires an array of distinct single-stranded probes of differing sequence immobilized on a substrate, which, when read in light of the Specification, requires "a structure that is made up of a substrate which includes the distinct nucleic acids immobilized at different and known locations on the surface of the support." (Br. 13.) Neither reference relied upon by the Examiner, Appellants assert, teaches or suggests "a method that employs a plurality of distinct nucleic acids immobilized on a surface of a *single* solid support." (*Id.*)

As noted by the Examiner (Answer 11-12), Conrad teaches a cocktail, *i.e.*, a mixture of probes (Conrad, col. 24, l. 53-col. 26, l. 3)), as well as methods of using the mixture (*Id.* at col. 28, l. 50-col. 29, l. 27). Appellants' argument that an array requires that different nucleic acids be immobilized at different and known locations on a solid support have been noted, but are not found to be convincing as Appellants do not point to where support for

the proposed claim interpretation that the nucleic acids are at a known position occurs in the Specification. Our review of the Specification finds that an array is defined as "a substrate having a planar surface on which is immobilized a plurality of distinct nucleic acid probes, in which each probe sequence on the array includes a constant domain and a complement variable domain." (Specification 5.) Thus, all that is required by the claim is that at least two different nucleic acid templates be immobilized on a solid support. Conrad teaches the use of cocktails (i.e., mixtures of two or more) probes, and given that teaching and the teaching of Dattagupta '363 that any substrate compatible with the chemistry may be used, such as nylon or paper, it would have been obvious to the ordinary artisan to immobilize two or more nucleic acid templates on a single template in order to synthesize a cocktail of probes as taught by Conrad. Finally, even if the claim were interpreted as Appellants propose, the method of claim 1 would have still been obvious to the ordinary artisan because, as noted by the Specification at page 5, arrays of nucleic acids are well known in the art and are commercially available.

Claims 10-15 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over the combination of Dattagupta '363 and Conrad as set forth above, as further combined with Dattagupta '899, designated as new grounds of rejection (Answer 13).

Dattagupta '363 and Conrad are relied upon as set forth above. According to the Examiner, "Dattagupta ['363] and Conrad do not teach using their nucleic acids as primer to make a population of target nucleic acids." (Answer 15).

Dattagupta '899 is cited because it teaches a similar method for making nucleic acid probes, that is "providing single-stranded probe nucleic acids, contacting with nucleic acids complementary to said constant domain under hybridization conditions whereby a template array of overhang comprising duplex nucleic acids is produced, wherein each overhang comprising duplex of said array comprises a double-stranded region and a single-stranded variable region overhang and using the duplex to produce probes (i.e. transcripts, Column 8, line 62-Column 9, line 16)." (*Id.*) Dattgupta '899 (citing col. 10, l. 51-col. 11, l.4) is also relied upon for teaching employing the probes as primers in a target generation step (*id.*).

The Examiner concludes:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to apply the probes of Dattagupta ['363] and Conrad in a primer extension reaction as taught by Dattagupta ['899] for the expected benefit of providing a quantity of amplification product as needed for sensitive detection as taught by Dattagupta ['899] (column 11, lines 3-4).

(*Id.* at 16.)

Appellants argue that claim 10 requires "an additional step of using the product mixture in the generation of a population of target nucleic acids *from an mRNA sample* by using the nucleic acid mixture a primer in a template dependent reaction, *where mRNA is the template*." (Reply Br. 16.) Appellants assert that neither Dattagupta '363 nor Conrad teaches that step, and that the deficiency is not remedied by Dattagupta '899.

According to Appellants, the Examiner relies on Figure 4 of Dattagupta '899, but no template array is produced in the method disclosed by the '899 patent (Reply Br. 17-18). In addition, Appellants assert that the

'899 patent does not teach or suggest a method in which the product is employed as a primer in a template dependent primer extension reaction in which mRNA is the template (Reply Brief 18).

Dattagupta '899 teaches at col. 10, l. 51-col. 11, l. 4:

> RNA mediated copying-This method creates a promoter site by using the transcript as a primer. The primer extension product acts as the transcribable signal. A sequence complementary to the RNA product is cloned into a single stranded phage vector e.g., M13. The transcript is allowed to react with such M13 DNA and the hybrid is then extended using a DNA polymerase and deoxynucleoside triphosphate some of which are labeled for the identification of the product. This process can also produce transcribable sequences for further amplification.
>
> An identical procedure can be followed via a synthetic oligonucleotide instead of a cloned DNA as the template for extension of the transcription product RNA primers.
>
> Promoter-containing DNA is hybridized with the product RNA, extended by using a DNA polymerase and then extended product is transcribed. Starting single stranded RNA does not transcribe. The final product is analyzed by capture with a specific immobilized probe. This process can be adjusted to make as much amplification as needed for sensitive analysis.

After careful review of the above teachings of the Dattagupta '899 patent, we agree with Appellants that the patent does not teach or suggest a method of using a mixture of nucleic acids as primers in a target generation step in which nucleic acids are produced from an mRNA sample. Thus, the rejection of claims 10-15 over the combination of Dattagupta '363, Conrad, and Dattagupta '899 is reversed. We do, however, raise other issues below, that the Examiner may want to consider in regard to the patentability of claims 10-15 upon return of the application.

Claim 23 stands rejected under 35 U.S.C. § 103(a) as being obvious over the combination of Dattagupta '363, Conrad, and Dattagupta '899.

Dattagupta '363 and Conrad are relied upon as above. According to the Examiner, "Dattagupta ['363] and Conrad both teach linear amplification but they do not teach strand-displacement amplification." (Answer 18.) The Examiner, asserts that as Dattagupta '899 teaches "a similar method for producing single stranded nucleic acids wherein the preferred method of linear amplification is strand displacement whereby multiple cycles of amplification are provided (Column 9, lines 58-67)," that "[i]t would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to apply the strand displacement of Dattagupta ['899] to the linear amplification of Dattagupta ['363] and Conrad based on the preferred teaching of Dattagupta ['363] (Column 9, lines 58-67." (Answer 18.)

In response, Appellants reiterate their arguments as to the combination of Dattagupta '363 and Conrad, asserting that Dattagupta '899 does not remedy the deficiencies of the combination. Thus, we affirm this rejection for the reasons set forth as to the rejection of claims 1-9, 21, and 22 over the combination of Dattagupta '363 and Conrad.

OTHER ISSUES

Claim 10 requires the steps of 1) generating a mixture of nucleic acids according to the method of Claim 1; and 2) employing said mixture of nucleic acids as primers in a target generation step in which nucleic acids are produced from said mRNA sample;. Claim 13 requires the additional steps of 1) contacting said set of target nucleic acids with an array of probe

nucleic acids under hybridization conditions; and 2) detecting the presence of target nucleic acids hybridized to probe nucleic acids of said array.

First, we have already concluded that the step of generating a mixture of nucleic acids according to the method of claim 1 is obvious over the combination of Dattagupta '363 and Conrad. Second, claims 10 and 13 appear to be drawn to steps that are used in gene expression analysis, discussed in the background of the invention, and, as noted by Appellants at page 15 of the Specification, "[g]ene expression analysis protocols are well known to those of skill in the art, and the populations of target nucleic acids produced by the subject methods find uses in many, if not all, of these protocols."

As an example of such a protocol, we point the Examiner's and Appellants' attention to Schena et al. ("Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes," *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 10614-10619 (1996)) and Srgoi et al. ("*In Vivo* Gene Expression Profile Analysis of Human Cancer Progression," *Cancer Research*, Vol. 59, pp. 5656-5661 (1999)).

Schena describes a gene expression analysis protocol, in which cDNA is made from mRNA using two probes (which reads on a mixture of nucleic acids, which could be generated using the method of claim 1). Schena also uses a micro array to detect the presence of target nucleic acids hybridized to probe nucleic acids of said array.

Sgroi teaches a method of gene expression profile analysis, in which mRNA was reverse transcribed using random hexamers (which reads on a mixture of nucleic acids, which could have been generated by the method of

claim 1), and also teaches the use of a microarray to detect the presence of target nucleic acids hybridized to probe nucleic acids of said array.

CONCLUSION

As the Examiner has set out a prima facie case of obviousness as to claims 1-9, 21, and over the combination of Dattagupta '363 and Conrad, and as to claim 23 over the combination of Dattagupta '363, Conrad, and Dattagupta '899, those rejection are affirmed. We are compelled to reverse the rejection of claims 10-15 under 35 U.S.C. § 103(a) as being obvious over the combination of Dattagupta '363, Conrad, and Dattagupta '899. We do, however, raise other issues the Examiner may wish to consider upon return of the application regarding the patentability of claims 10-15.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv)(2006).

AFFIRMED-IN-PART

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